APRW -31

Effect of *in ovo* Betaine-Hydrochloride Supplementation on Hatchability, Chick Quality and Organ Development of Broiler Chicks

D.A. Tanimowo and O.G. Longe
Department of Animal Science, University of Ibadan, Ibadan, Nigeria
Corresponding author: D.A. Tanimowo; E-mail:Edamilolatanimowo@yahoo.com; Tel. 07035571461

Abstract
Two trials were carried out to assess the effect of *in ovo* supplementation of betaine-hydrochloride (BH) on the hatchability, chick quality and organ development of broiler chicks. Trial one was carried out using 160 Marshall broiler chicks at day 18 of incubation. Treatments included: un-injected control, 0.9% saline, 0.9% saline + 5 mgBH, 0.9% saline + 10mg BH and 0.9% saline +15mg BH. Trial 2 was carried out using 266 Marshall broiler chicks at day 18 of incubation. Treatments were un-injected control, 0.9% saline, 0.9% saline + 5mgBH, 0.45% saline + 10 mgBH and 0.23% saline + 15mg BH. Parameters observed were hatchability, chick quality (length) and chick weight. In trial two, in addition, visceral organ development was assessed. *In ovo* BH influenced chick length and rectal temperatures ($p<0.05$) in both trials. Organ weights were also significantly ($p<0.05$) influenced by BH supplementation *in ovo*, and these effects were sustained 7 days after hatch.

Keywords: *In ovo*, betaine-hydrochloride, broiler chicks, chick quality, chick development

Introduction

Glycogen stored in the liver and muscles of embryos is the main energy source during the hatching process (Moran, 2007). However at this stage of incubation, glycogen is utilized in a greater amount in order to meet the high energy demand of the hatching process, and consequently, glycogen reserves in the embryo are significantly depleted at the end of incubation. Weight loss between hatching and removal from the hatcher is approximately 0.18g/h while fasting (Halevy *et al*., 2000). Several alternatives have been proposed to improve performance during this initial phase such as *in ovo* feeding (Uni and Ferket, 2004). This method is based on the fact that neonatal birds naturally consume the embryonic amniotic fluid towards hatch (Romanoff, 1960). Therefore, the addition of a nutrient solution to the embryonic amniotic fluid delivers essential nutrients into the embryo intestine. Betaine is distributed widely in animals, plants, and micro-organisms. In addition to dietary sources, betaine can be produced through the irreversible oxidation of choline. As an osmolyte, betaine protects cells by regulating cell volume, stabilizing proteins, and enzymes from environmental stress (Craig, 2004). Moreover, betaine has been discussed for its role in saving methionine and choline (Fernandez-Figares *et al*., 2002), making methionine more available for protein synthesis, and making choline more available for lipid metabolism.

Thus this study proposed that *in ovo* supplementation of betaine in hydrochloride form could improve energy storage, consequent hatchability, chick quality and organ development.

Materials and methods

A total of 160 fertile 18days incubated eggs of comparable weight produced by a flock of 45-week-old hens from a meat-type breeder (Marshal), were used in the first trial to observe the effect of the combination of betaine-hydrochloride (BH) and 0.9% saline on hatchability and chick quality.The eggs were examined and selected after candling. The *in ovo* treatments were two control treatments: non-injected (negative control, NC), and injected with 0.9% sterilized saline (positive control, PC) and three other treatments in which sterilised saline containing dissolved quantities of 5, 10 and 15mg of BHwere injected. The wide end of the eggshell was disinfected with 70% isopropyl alcohol where-upon an injection hole was pierced. The 1 mL of solution was injected into the yolk sac using a 25 mm x 21-G needle. After injection, the hole was sealed with cellophane tape and the eggs were returned to the incubators. Percent hatchability was calculated on the basis of the number of hatched chicks as a percentage of the *in ovo* treated eggs per replicate.

At the end of incubation, chicks were weighed. Chick quality (chick length (cm) and rectal temperature (°C)) was also determined.In the second trial to assess *in ovo* supplementation of BH and varying saline concentrations, a total of 266 eggs were evenly assigned to 2 hatching trays divided into individual hatching sections. Eggs were treated and *in ovo* procedure carried out as described for experiment 1 and all parameters observed in experiment 1 were repeated. At the end of the incubation period, six chicks were randomly selected

from each experimental treatment. The weight of the chick was determined, and visceral organs removed, weighed and relative weights calculated as a percentage of the live weight. This was also repeated after 7days (using 4 birds per treatment).

Data from trials 1 and 2 were analyzed separately using one-way ANOVA and means separation carried out using least significant difference (LSD). All percent data were transformed using arcsine square root percentage transformation before analysis using SPSS version 2008.

Results and Discussion

It was observed that using 0.9% saline solution as a buffer at 10 mg and 15 mg greatly reduced hatchability (Table 1). Even though the osmolarity for both solutions were within the range described by Uni and Ferket (2003) as the acceptable range, though theirs was stated for osmolality. The negative effect could be as a result of the difference between osmolarity and osmolality, since osmolality takes into consideration practical interactions between solutes. When this was corrected in the trial 2, by varying the saline concentrations, there was a markedimprovement in hatchability. Betaine-hydrochloride supplementation at 15 mg had the highest values, while the un-injected control had the lowest values. Betaine as an osmolyte has been effective as an osmolyte, by protecting cells from stress (Craig, 2004), even though the higher osmolarity in trial 1 could not be ameliorated by the presence of betaine.

Table 1: Hatchability and chick quality of broiler chicks administered betaine-hydrochloride *in ovo* using iso-saline/varying saline levels as diluent

Trial one						
	NC	PC	BH5mg	BH10mg	BH15mg	SEM
Osmolarity* (mMol)		308.0	405.5	503.3	601.0	
Hatchability	93.8	71.9	100.0	59.5	48.5	
Chick weight	38.9^{a}	39.8^{a}	40.5^{ab}	40.5^{ab}	41.0^{b}	0.31
Chick length	19.5^{a}	19.1^{ab}	18.9^{bc}	18.6^{c}	18.6^{c}	0.06
Rectal temp	38.5^{a}	38.8^{a}	39.4^{b}	39.3^{b}	39.4^{b}	0.08
Trial two						
	NC	PC	BH5mg	BH10mg	BH15mg	SEM
Osmolarity (mMol)*		308.00	405.50	349.30	369.95	
Hatchability	90.30	92.65	94.20	92.65	96.55	1.41
Chick weight	39.73	40.07	40.20	40.07	40.39	0.14
Chick length	19.15^{a}	19.45^{ab}	19.38^{ab}	19.57^{b}	19.56^{b}	0.06
Rectal temp	40.00^{a}	40.12^{a}	40.56^{b}	39.65^{a}	39.95^{a}	0.04

NC- un-injected control; PC- positive control (0.9% saline); BH 5mg- betaine-hydrochloride (5 mg+0.9% saline); BH10 mg- betaine-hydrochloride (10 mg+0.45% saline); BH15 mg- betaine-hydrochloride (15 mg+0.23% saline)
* Osmolarity was calculated from the molecular weights of solutes.
$^{ab\ c}$ Means in a column within genotype and ATC are different

Also, since betaine is an ideal methyl donor, it would be required in methylation reactions for methyl donation into the gluconeogenic pathway; which is a major pathway at hatching. There was a significant effect of supplementation observed in trial 1 with betaine supplementation having higher values compared with the controls, with weight differences as much as 5% higher when comparing 15 mg and uninjected controls. There was no difference observed in trial 2, but values still showed numerically higher weights compared with the un-injected controls. Kadam *et al*. (2013) also observed that *in ovo* betaine supplementation did improved hatch weight at 100 mg compared with the un-injected control, though other betaine levels were significantly lower. This could be as a result of the fact that in that study Milli Q water was used as the buffer not saline.

Relative organ weights at hatch day are presented in table 2. It was observed that *in ovo* supplementation had a significant effect on all the organs except the small intestine. Yolk sac weights were not significantly influenced by supplementation. Pectoral muscle weights were observed to be highest on the un–injected control birds. This did not reflect the improvements in weights observed at hatch. It could however, be that supplementation improved fluid retention in the supplemented birds. It was also observed that supplemented birds had numerically larger yolk sac compared with the uninjected birds, which could have resulted in the larger weights observed in the injected birds. Dos Santos *et al.* (2010) observed numerically larger relative yolk sac weights in chicks supplemented with various nutrients compared with the un-supplemented chicks. They were of

the opinion that the supplementation of nutrients into the amnion helps reduce the need to use yolk content as an energy source, and may explain the higher yolk percentage in supplemented birds. Their results however showed no significant effect of *in ovo* supplementation on liver and intestine weights.

Table 2: Relative organ weights of *in ovo* betaine supplemented broilers at hatch day

Organ		NC	PC	BH5mg	BH10mg	BH15mg	SEM
Liver	D0	3.1^{ab}	3.3^{a}	2.9^{b}	2.8^{b}	3.0^{ab}	0.06
	D7	3.8	4.3	3.1	3.5	4.0	0.19
Heart	D0	0.8^{a}	0.9^{a}	0.9^{a}	0.8^{a}	0.7^{b}	0.02
	D7	1.1^{a}	0.9^{ab}	0.8^{ab}	0.6^{b}	0.8^{ab}	0.06
Pectorals	D0	3.6^{a}	3.1^{ab}	3.1^{ab}	2.5^{b}	3.0^{ab}	0.12
	D7	8.4^{ab}	8.3^{b}	8.2^{b}	9.4^{ab}	9.7^{a}	0.24
Kidney	D0	0.7^{ab}	0.8^{a}	0.7^{ab}	0.7^{ab}	0.7^{b}	0.02
	D7	1.2^{a}	1.1^{a}	0.9^{b}	1.2^{a}	1.1^{a}	0.04
Small intestine	D0	3.9	4.1	3.9	3.7	4.3	0.14
	D7	9.3^{a}	8.4^{ab}	8.0^{b}	8.9^{ab}	8.2^{ab}	0.19
Yolk	D0	7.0	10.0	7.4	8.7	8.0	

NC- un-injected control; PC- positive control (0.9% saline); BH 5mg- betaine-hydrochloride (5 mg+0.9% saline); BH10 mg- betaine-hydrochloride (10 mg+0.45% saline); BH15 mg- betaine-hydrochloride (15 mg+0.23% saline)
$^{a\,b\,c}$ Means in a column within genotype and ATC are different

At the 7th day (Table 2) of age, results showed that betaine supplementation had no effect ($p>0.05$) on liverweights. It was however observed that un-injected controls had significantly larger hearts compared with supplemented chicks, while pectoral muscle weights wee highest at 10mg and 15mg betaine supplementation levels. Kidney and small intestine weights were also significantly reduced in the betaine supplemented chicks. According to Nguyen *et al.* (2013), the gastrointestinal tract consumes up to 25% of the total body oxygen consumption utilizing a large part of total ingested energy, with around 35% of this energy associated with the maintenance of ionic homeostasis (Jessop, 2000). Betaine is believed to reduce energy costs of the ion pump when acting as an organic osmolyte. They observed that inclusion of betaine in diets of broiler chicks significantly improved heat production and heat increment. This could reflect in higher performance in broilers. An increase in intestinal weight and length has been associated with need to increase surface area for digestion.Dos Santos *et al*. (2010) did not observe any significant improvement in pectoral muscle weight when inoculating eggs with various nutrients. Though Hamadani *et al*. (2013) stated that at the time of hatch, the small intestine of the *in ovo* supplemented bird is a functional stage similar to that in conventionally fed 2-day old chicks and that several experiments with offspring from young maternal flocks demonstrated that *in ovo* feeding increases hatchling weights by 5% over controls and elevates relative breast-muscle size. These weight advantages were sustained up to 25 days.

Conclusion

From this study, it was observed that *in ovo* betaine supplementation improved hatchability, chick quality and hatch day weights of broiler chicks. There was also significant effect on relative organ weights and organ development of supplemented chicks, with marked improvement in pectoral muscle mass and development observed at 10mg. Further studies should involve assessing *in ovo* betaine effects at substrate level, as well as gut morphological development as indicators for intestinal response to its administration.

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